Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

City of Nashville
WPCP
OUTFALL 001

Chronic Biomonitoring Report

70464

Ceriodaphnia dubia
Pimephales promelas

October 23, 2018

Approved by: Joshua Reed

Bio-Aquatic Testing, Inc. • *2501 Mayes Rd. Ste. 100* • *Carrollton, Texas* • *75006*

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***HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

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TOXICITY TEST REPORT - Chronic

Client:	Nashville, City of	Sample:	001
Facility:	WPCP	Laboratory Number:	70464
Permit No.	GA0039365	Date:	October 23, 2018

Ceriodaphnia dubia **passed** survival and reproduction testing requirements. *Pimephales promelas* **passed** survival and growth testing requirements.

SAMPLE COLLECTION:

Composite effluent samples from the City of Nashville, WPCP, were received on October 23, 2018, October 26, 2018, and October 27, 2018. Effluent samples were collected from Outfall 001 by facility personnel.

The effluent samples were analyzed for total residual chlorine using the Hanna Ion Specific Meter #711 and contained <0.10 mg/L, <0.10 mg/L, and <0.10 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily.

TEST PROCEDURES:

Ceriodaphnia dubia

EPA METHOD: 1002

The seven-day (three brood) Chronic *Ceriodaphnia dubia* survival and reproduction test was initiated at 13:33 hours on October 23, 2018. Five effluent concentrations of 12.5%, 25%, 50%, 69% and 100% were prepared using synthetic water as dilution water. The test was set up with 30mL plastic cups containing 15mL of test solution or control dilution water. Each effluent concentration or control dilution water included ten replicate cups with one organism in each cup. The control was conducted concurrently with the test. Test organisms were less than 24-hour old laboratory cultured neonates. Neonates were introduced into the test solutions using a blocking design. The test was renewed daily with newly prepared solutions. Food consisting of a half-milliliter suspension of the green algae, *Selenastrum capricornutum,* and YTC was added to the test solutions each day. The test proceeded for seven days or until 60% of the females in the control had three broods. Data on survival and number of young produced per female were collected daily. The test ended at 08:11 hours on October 30, 2018. Survival and reproduction data were statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Ceriodaphnia dubia

Fisher's Exact test on *Ceriodaphnia dubia* survival test data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

REPRODUCTION:

Ceriodaphnia dubia

The *Ceriodaphnia dubia* reproduction data were normally distributed at the alpha level of 0.01 (13.277) using the Chi-square test for normality. Reproduction data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Using ANOVA and Dunnett's Test, *Ceriodaphnia dubia* reproduction data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

TEST PROCEDURES:

EPA METHOD: 1000

Pimephales promelas

The seven-day Chronic *Pimephales promelas* survival and growth test was initiated at 13:37 hours on October 23, 2018. Five effluent concentrations of 12.5%, 25%, 50%, 69% and 100% were prepared using synthetic water as dilution water. The test was set up with 450mL plastic cups containing 250mL of test solution as test chambers. Each concentration consisted of five replicate chambers containing eight organisms each, giving a total of 40 (forty) per treatment. The control test was conducted concurrently with the test. Test organisms were laboratory-cultured *Pimephales promelas* larvae less than 24-hours old. The number of surviving larvae and water quality parameters in the old test solutions were recorded after each 24-hour period. The test was renewed daily with fresh solutions. Surviving larvae in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for seven days.

At the end of the test, all organisms were sacrificed, dried, and weighed. Data on surviving organisms and water quality were collected. The test ended at 11:15 hours on October 30, 2018. Survival and growth (weight) were statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Pimephales promelas

The non-parametric Steel's Many-One Rank test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

GROWTH:

Pimephales promelas

The *Pimephales promelas* growth data were normally distributed at the alpha level of 0.01 (0.900) using Shapiro Wilk's test for normality. Growth data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Using ANOVA and Dunnett's Test on *Pimephales promelas* growth data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 100% Effluent

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

Chronic ***Ceriodaphnia dubia***

Client: Nashville, City of WPCP

Permit Number: NPDES GA0039365

Sample Type: Composite

Outfall Name: 001

Receiving Water Name:

Lab ID: 70464

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light, 8 hours dark

Dilution Water: synthetic

Begin Date: 10/23/2018

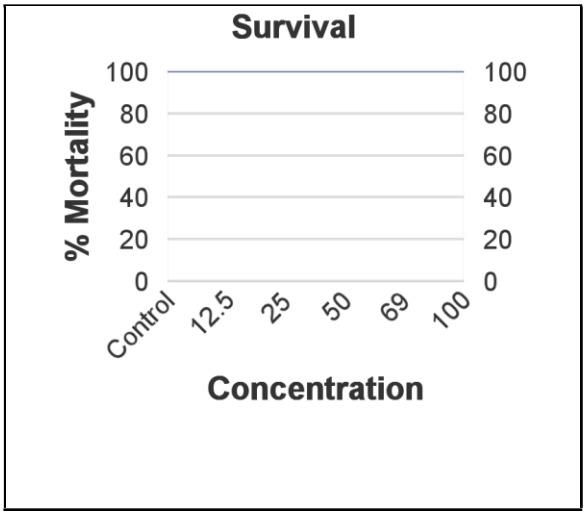
End Date: 10/30/2018

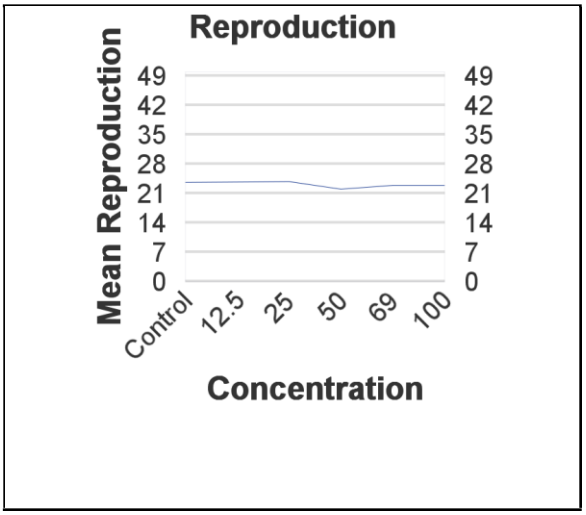
Test Start Time: 13:33 **Test End Time:** 08:11

SURVIVAL AND REPRODUCTION TABLE

FEMALE #	Control	12.5	%	25	%	50	%	69	%	100	%
1	23	24		26		21		21		24	
2	24	25		26		20		25		27	
3	25	24		17		21		28		21	
4	23	22		23		14		26		25	
5	20	18		29		17		16		26	
6	23	21		16		21		20		28	
7	23	22		29		24		23		23	
8	25	31		27		27		24		18	
9	27	25		22		29		26		17	
10	23	25		23		26		20		20	
Surv.Mean	23.6	23.7		23.8		22.0		22.9		22.9	
C.V%	7.7	14.3		19		20.8		15.8		16.5	
Total Mean	23.6	23.7		23.8		22.0		22.9		22.9	
Var	3.377	11.566		20.622		21.111		13.211		14.322	
Std.Dev.	1.837	3.4		4.541		4.594		3.634		3.784	
Max	27	31		29		29		28		28	
Min	20	18		16		14		16		17	

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Survival and Reproduction

Control

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	4	A
10/27	4	5	6	4	3	5	6	3	A	5
10/28	8	A	6	9	5	6	8	7	9	7
10/29	A	9	A	A	A	A	A	A	A	11
	12	14	12	13	8	11	14	10	13	23
10/30	11	10	13	10	12	12	9	15	14	A
	23	24	25	23	20	23	23	25	27	23
10/31										

Mean: 23.60 **CV%** 7.70
Var. 3.38 **Max** 27
Std.Dev. 1.84 **Min** 20

12.5

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	A	A
10/27	5	5	4	3	4	5	6	5	5	4
10/28	7	A	10	5	6	A	7	11	7	5
10/29	A	9	A	A	A	7	A	A	A	A
	12	14	14	8	10	12	13	16	12	9
10/30	12	11	10	14	8	9	9	15	13	16
	24	25	24	22	18	21	22	31	25	25
10/31										

Mean: 23.70 **CV%** 14.30
Var. 11.57 **Max** 31
Std.Dev. 3.40 **Min** 18

25

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	A	A
10/27	5	5	6	5	5	5	5	6	5	2
10/28	6	A	3	A	A	A	A	A	8	8
10/29	A	8	8	6	9	11	11	10	A	13
	11	13	17	11	14	16	16	16	13	23
10/30	15	13	A	12	15	A	13	11	9	A
	26	26	17	23	29	16	29	27	22	23
10/31										

Mean: 23.80 **CV%** 19.00
Var. 20.62 **Max** 29
Std.Dev. 4.54 **Min** 16

50

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	A	A
10/27	4	5	5	3	6	5	5	6	5	6
10/28	7	6	7	A	5	A	A	10	11	9
10/29	A	A	A	3	6	7	7	A	13	11
	11	11	12	6	17	12	12	16	29	26
10/30	10	9	9	8	A	9	12	11	A	A
	21	20	21	14	17	21	24	27	29	26
10/31										

Mean: 22.00 **CV%** 20.80
Var. 21.11 **Max** 29
Std.Dev. 4.59 **Min** 14

69

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	A	A
10/27	5	5	3	5	5	5	4	6	5	A
10/28	7	7	10	8	A	6	A	8	10	9
10/29	9	A	A	A	11	A	8	A	11	11
	21	12	13	13	16	11	12	14	26	20
10/30	A	13	15	13	A	9	11	10	A	A
	21	25	28	26	16	20	23	24	26	20
10/31										

Mean: 22.90 **CV%** 15.80
Var. 13.21 **Max** 28
Std.Dev. 3.63 **Min** 16

100

Date	1	2	3	4	5	6	7	8	9	10
10/24	A	A	A	A	A	A	A	A	A	A
10/25	A	A	A	A	A	A	A	A	A	A
10/26	A	A	A	A	A	A	A	A	A	A
10/27	4	5	5	4	5	A	5	4	A	6
10/28	9	9	6	5	8	8	7	4	A	3
10/29	11	A	A	A	A	9	A	A	8	11
	24	14	11	9	13	17	12	8	8	20
10/30	A	13	10	16	13	11	11	10	9	A
	24	27	21	25	26	28	23	18	17	20
10/31										

Mean: 22.90 **CV%** 16.50
Var. 14.32 **Max** 28
Std.Dev. 3.78 **Min** 17

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Nashville, City of - WPCP** Lab ID: **70464** Culture No.: Bio102318-325

TEST INSTRUCTIONS:

ORGANISMS ADDED: Date: 10-23-18 Time: 1333 Technician: KM

Photo Period 16hr Light/8hr dark

RANDOMIZATION: SC-10 19

Dilution: Control

	DATE/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr	10-24-18 AP 1346	A	—	—	—	—	—	—	—	—	A
48Hr	10-25-18 AP 1140	A	—	—	—	—	—	—	—	—	A
72Hr	10-26-18 KB 1025	A	—	—	—	—	—	—	A	4	A
96Hr	10-27-18 KB 1412	4	5	6	4	3	5	6	3	A	5
5 days	10-28-18 KB 0953	6	A	6	9	5	6	8	7	9	7
6 days	10-29-18 SF 1108	3	9	A	—	—	—	—	A	6	11
7 days	10-30-18 SF 0811	8	10	13	10	12	12	9	15	8	A
8 days											

Dilution: 12.5 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A	—	—	—	—	—	—	—	—	A
48Hr	A	—	—	—	—	—	—	—	—	A
72Hr	A	—	—	—	—	—	—	—	—	A
96Hr	5	5	4	3	4	5	6	5	5	4
5 days	7	A	10	5	6	A	7	11	7	5
6 days	A	9	A	—	A	7	A	—	—	A
7 days	12	11	10	14	8	9	9	15	13	16
8 days										

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Nashville, City of - WPCP** Lab ID: **70464** Culture No.:

TEST INSTRUCTIONS:

Dilution: 25 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	5	5	6	5	5	5	5	6	5	A
5 days	6	A	3	A				A	8	8
6 days	A	7	8	6	9	11	11	10	A	13
7 days	15	13	A	12	15	A	13	11	9	A
8 days										

Dilution: 50 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	4	5	5	3	6	5	5	6	5	6
5 days	7	6	7	A	5	A	A	10	11	9
6 days	A		A	3	6	7	7	A	13	11
7 days	10	9	9	8	A	9	12	11	A	A
8 days										

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Nashville, City of - WPCP** Lab ID: **70464** Culture No.:

TEST INSTRUCTIONS:

Dilution: 69 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	5	5	3	5	5	5	4	6	5	A
5 days	7	7	10	8	A	6	A	8	10	9
6 days	9	A	A	A	11	A	8	A	11	11
7 days	A	13	15	13	A	9	11	10	A	A
8 days										

Dilution: 100 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A									A
96Hr	4	5	5	4	5	3	5	4	A	6
5 days	9	9	6	5	8	5	7	4	A	3
6 days	11	A			A	9	A	A	8	11
7 days	A	13	10	16	13	11	11	10	9	A
8 days										

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

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Chronic **CERIODAPHNIA DUBIA** **SURVIVAL AND REPRODUCTION**

Client: **Nashville, City of - WPCP** Lab ID: **70464** Culture No.:

TEST INSTRUCTIONS:

Test Temperatures

	0Hr	24Hr		48Hr		72Hr		96Hr		5 days		6 days		7 days
	new	old	new	old	new	old	new	old	new	old	new	old	new	old
Control	25.1	25.0	24.7	25.0	24.7	24.8	25.0	25.0	25.1	24.7	24.9	24.7	25.0	24.9
12.5														
25														
50														
69														
100														
TIME/DATE TECH	10-23-18 ICB 1333	10-24-18 AP	1346	10-25-18 AP	1140	10-26-18 ICB	1026	10-27-18 ICB	1412	10-28-18 ICB	1453	10-29-18 SF	1108	10-30-18 SF	0811
IR GUN ID #	008	008		008		008		008		008		008		008	

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

Chronic *Pimephales promelas*

Client: Nashville, City of WPCP
Lab ID: 70464
Permit Number: NPDES GA0039365
Test Temperature (oC): 25 ± 1
Outfall Name: 001 **Sample Type:** Composite
Photo Period: 16 Hours Light 8 Hours Dark
Receiving Water Name:

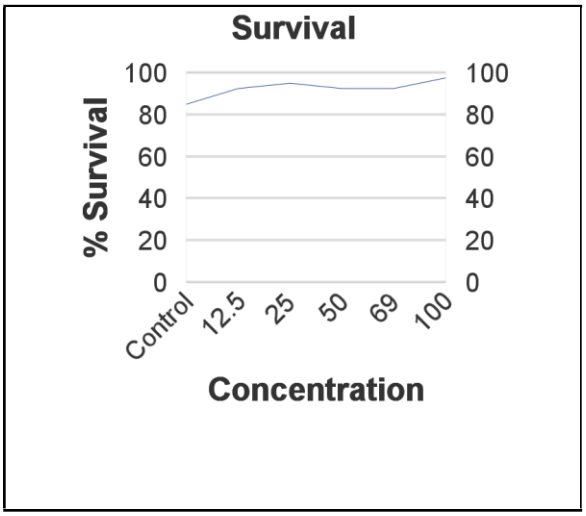
Test Start Time: 13:37 Test End Time: 11:15
Begin Date: 10/23/2018
End Date: 10/30/2018

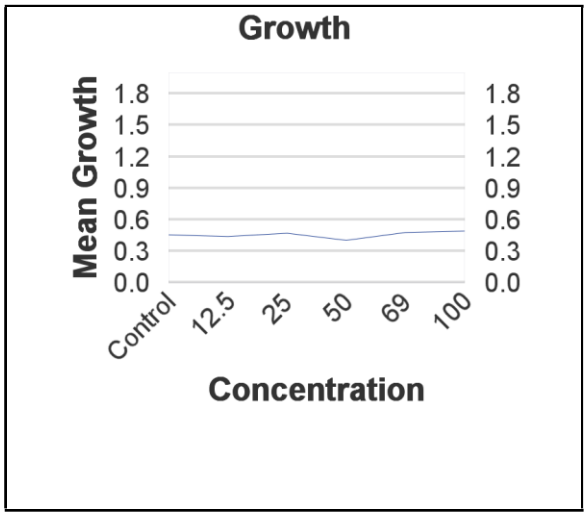
SURVIVAL

Effluent Concentration		Number Of Alive 10/23	10/24	10/25	10/26	10/27	10/28	10/29	10/30	Avg% Surv.
Control	A	8	8	8	8	7	7	7	6	85.0%
	B	8	8	8	7	7	7	7	7	
	C	8	8	8	8	8	6	6	6	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	7	7	7	7	
12.5	A	8	8	8	8	8	8	8	8	92.5%
	B	8	8	8	7	7	7	7	7	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	7	6	6	6	6	
25	A	8	8	8	7	7	7	7	7	95.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	7	7	7	7	7	
50	A	8	8	8	8	8	8	8	8	92.5%
	B	8	8	8	8	8	8	8	7	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	8	8	6	6	

Effluent Concentration		Number Of Alive 10/23	10/24	10/25	10/26	10/27	10/28	10/29	10/30	Avg% Surv.
69	A	8	8	8	7	7	7	7	7	92.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	7	7	7	7	7	
	D	8	8	8	7	7	7	7	7	
	E	8	8	8	8	8	8	8	8	
100	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	7	7	
	E	8	8	8	8	8	8	8	8	
	A									
	B									
	C									
	D									
	E									

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **70464**

Client: **Nashville, City of** Facility **WPCP** Outfall:001

Sample Type Composite

TEST INSTRUCTIONS:

Culture No. : P1-18-295 Photo Period: 16hr light, 8hr dark **RANDOMIZATION:** **SC-5 1**

Dilution: Control 12.5 25 50

	DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	10-23-18 MMM 1337	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
24Hr	10-24-18 MMM 918	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
48Hr	10-25-18 JLM 1145	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
72Hr	10-26-18 SKF 1110	8	7,0	8	—	—	8	7,1	8	8	7,1	7,1	8	—	—	7,1	8	—	—	—	—
96Hr	10-27-18 MMM 1026	7,0	7	8	8	7,1	8	7	8	8	6,1	7	8	—	—	7	8	—	—	—	—
5 days	10-28-18 MMM 1029	7	7	6,2	8	7	8	7	8	8	6	7	8	—	—	7	8	—	—	—	—
6 days	10-29-18 MMM 1029	7	7	6	8	7	8	7	8	8	6	7	8	—	—	7	8	—	—	—	6,2
7 days	10-30-18 MMM 1115	6,0	7	6	8	7	8	7	8	8	6	7	8	—	—	7	8	7,1	8	8	6

Dilution: 69 100

	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	8	—	—	—	—	8					8									
24Hr	8	—	—	—	—	8	—	—	—	—										
48Hr	8	—	—	—	—	8	—	—	—	—										
72Hr	7,1	8	7,1	7,1	8	8	—	—	—	—										
96Hr	7	8	7	7	8	8	—	—	—	—										
5 days	7	8	7	7	8	8	—	—	—	—										
6 days	7	8	7	7	8	8	—	—	7,1	8										
7 days	7	8	7	7	8	8	—	—	7	8										

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable sp

Chronic	Pimephales promelas SURVIVAL	Lab ID: **70464**

Client: **Nashville, City of** Facility **WPCP** Outfall:001 Sample Type Composite

TEST INSTRUCTIONS:

Test Temperatures

	0Hr	24Hr		48Hr		72Hr		96Hr		5 days		6 days		7 days
	new	old	new	old	new	old	new	old	new	old	new	old	new	old
Control	25.4	25.6	25.3	25.5	25.4	25.3	25.5	25.5	25.7	25.3	25.4	25.5	25.4	25.8
12.5		25.5												
25														
50														
69														
100														
TIME/DATE TECH	10-23-18 MMM 1337	10-24-18 MMM 918		10-25-18 JLM 1145		10-26-18 KB 1025 MMM 1020		10-27-18 MMM 1020		10-28-18 MMM 1029		10-29-18 MMM 1011		10-30-18 MM 1024
IR GUN ID #	016	016		016		016		016		016		016		016

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

2nd Organism **BIO-AQUATIC TESTING, INC.** **TOXICITY TEST**

Chronic *Pimephales promelas*

Client: Nashville, City of WPCP **Lab ID: 70464**

Permit Number: GA0039365

Sample Type: Composite **Outfall Name:** 001

Receiving Water Name:

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	6	2.838	0.355	0.473
B	8	7	3.735	0.467	0.534
C	8	6	3.245	0.406	0.541
D	8	8	3.928	0.491	0.491
E	8	7	4.388	0.549	0.627

Mean	C.V. %
0.453	16.6

SN Mean	SN C.V. %
0.533	11.2

12.5

	ON	Wt.	Avg.
A	8	3.690	0.461
B	8	3.650	0.456
C	8	3.323	0.415
D	8	3.522	0.440
E	8	3.327	0.416

Mean	C.V. %
0.438	5.0

25

	ON	Wt.	Avg.
A	8	2.950	0.369
B	8	4.399	0.550
C	8	3.914	0.489
D	8	3.492	0.437
E	8	3.988	0.499

Mean	C.V. %
0.469	14.7

50

	ON	Wt.	Avg.
A	8	3.185	0.398
B	8	3.677	0.460
C	8	2.721	0.340
D	8	4.110	0.514
E	8	2.362	0.295

Mean	C.V. %
0.401	21.9

69

	ON	Wt.	Avg.
A	8	3.564	0.446
B	8	4.432	0.554
C	8	4.090	0.511
D	8	3.330	0.416
E	8	3.610	0.451

Mean	C.V. %
0.476	11.7

100

	ON	Wt.	Avg.
A	8	4.157	0.520
B	8	3.929	0.491
C	8	3.557	0.445
D	8	4.229	0.529
E	8	3.689	0.461

Mean	C.V. %
0.489	7.4

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

Note: ON stands for original number per replicate, while SN refers to the number surviving after test completion.

BIO-AQUATIC TESTING, INC. TOXICITY TEST

Chronic — Pimephales promelas — Lab ID: 70464

Client: Nashville, City of - WPCP

Balance: Radwag BAL-007

Begin Date: 10/23/2018 End Date: 10/30/2018 Organism: Pimephales promelas

Analyst: JF

Weigh Date: 11-03-18

Date/Time placed in Oven: 10-30-18 / 1545

Date/Time removed from Oven: 10-31-18 / 1600

Control

	Qty.	Wt.
A	6	2.838
B	7	3.735
C	6	3.245
D	8	3.928
E	7	4.388

12.5 %

	Qty.	Wt.
A	8	~~2.838~~ 3.690
B	7	3.650
C	8	3.323
D	8	3.522
E	6	3.327

25 %

	Qty.	Wt.
A	7	2.950
B	8	4.399
C	1	3.914
D	1	3.492
E	7	3.988

50 %

	Qty.	Wt.
A	8	~~2.302~~ 3.185
B	7	~~4.110~~ 3.677
C	8	2.721
D	8	4.110
E	6	2.362

69 %

	Qty.	Wt.
A	7	3.564
B	8	4.432
C	7	4.090
D	7	3.330
E	8	3.610

100 %

	Qty.	Wt.
A	8	4.157
B	1	3.929
C	1	3.557
D	7	4.229
E	8	3.689

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

APPENDIX A

STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. The fertilization organisms were calculated according to EPA-600-R-95-136 or EPA-600-R-12-022, dependent upon the species. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Mysidopsis bahia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The fertilization data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation or TST methodology may also be used.

Cerio Repro
File: 70464.cdr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	6	9	27	15	3

Calculated Chi-Square goodness of fit test statistic = 4.0747
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Cerio Repro
File: 70464.cdr Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 7.46

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

Cerio Repro
File: 70464.cdr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	23.750	4.750	0.338
Within (Error)	54	757.900	14.035	
Total	59	781.650		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho: All equal

Cerio Repro
File: 70464.cdr Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	23.600	23.600		

2	12.5	23.700	23.700	-0.060
3	25	23.800	23.800	-0.119
4	50	22.000	22.000	0.955
5	69	22.900	22.900	0.418
6	100	22.900	22.900	0.418

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Cerio Repro
File: 70464.cdr Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	con	10			
2	12.5	10	3.870	16.4	-0.100
3	25	10	3.870	16.4	-0.200
4	50	10	3.870	16.4	1.600
5	69	10	3.870	16.4	0.700
6	100	10	3.870	16.4	0.700

FAthead growth
File: 70464.ppg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.092

W = 0.977

Critical W (P = 0.05) (n = 30) = 0.927
Critical W (P = 0.01) (n = 30) = 0.900

Data PASS normality test at P=0.01 level. Continue analysis.

FAthead growth
File: 70464.ppg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 7.63

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

FAthead growth
File: 70464.ppg Transform: NO TRANSFORMATION

70464

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.025	0.005	1.289
Within (Error)	24	0.092	0.004	
Total	29	0.117		

Critical F value = 2.62 (0.05,5,24)
Since F < Critical F FAIL TO REJECT Ho: All equal

FAthead growth
File: 70464.ppg Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	coon	0.454	0.454		
2	12.5	0.438	0.438	0.408	
3	25	0.469	0.469	-0.387	
4	50	0.401	0.401	1.331	
5	69	0.476	0.476	-0.561	
6	100	0.489	0.489	-0.907	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

FAthead growth
File: 70464.ppg Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	coon	5			
2	12.5	5	0.093	20.4	0.016
3	25	5	0.093	20.4	-0.015
4	50	5	0.093	20.4	0.052
5	69	5	0.093	20.4	-0.022
6	100	5	0.093	20.4	-0.036

Bio-Aquatic Testing, Inc.

FRESH WATER TEST SETUP FORM

Client: Nashville, City of

Permit GA0039365

Facility: WPCP

Lab Number 70464

Outfall Name: 001

Number of samples 3

Dilution Water: Synthetic Lab

Receiving Water Name:

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	10/23/18	10:40	10/21/18	10/22/18	10:00	08:00
2	10/26/18	08:30	10/23/18	10/24/18	10:45	08:30
3	10/27/18	15:20	10/25/18	10/26/18	09:20	08:15

Type of Test(s)

Ceriodaphnia dubia	Chronic
Pimephales promelas	Chronic

Dilution Water

Sample #	Hardness As mg/L $CaCO_3$	Alkalinity as mg/L $CaCO_3$
1	140	55
2	140	55
3	140	55

Start Sx # 1 Date: 10/23/2018

Renew Sx # 1 Date: 10/24/2018

Renew Sx # 1 Date: 10/25/2018

Renew Sx # 2 Date: 10/26/2018

Renew Sx # 2 Date: 10/27/2018

Renew Sx # 3 Date: 10/28/2018

Renew Sx # 3 Date: 10/29/2018

Test Start Date: 10/23/2018

Test End Date: 10/30/2018

Ceriodaphnia dubia Test Set Up: 10 Reps & 1 Organisms per Rep

Pimephales Test Set Up: 5 Reps & 8 Organism per Rep

Concentrations: 12.5 25 50 69 100 % LF % 69

Test Chemistry on these dilutions: 12.5 25 50 69 100

Samples received by:

○ Greyhound	○ UPS Next Day	○ Delta Dash	○ Delta
○ Pony Express	○ Client Delivered	○ Southwest Airlines	○ DHL
◉ Federal Express	○ American Airlines	○ Bio Pick Up	

Other:

BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

Client: Nashville, City of **Lab ID:** 70464

Facility: WPCP **Outfall:** 001

Dilution Water(s): Synthetic Lab **Test Date:** October 23, 2018

EFFLUENT PARAMETERS

Effluent Sample #	Received		Residual Cl_2 (mg/L)	DeChlor (ml/L)[1]	Ammonia (mg/L)	Analyst Initials	Temp. Received
	Date	Time					
1	10/23/18	10:40	<0.10	N/A	<0.25	DF	3.3
2	10/26/18	08:30	<0.10	N/A	<0.25	DF	2.1
3	10/27/18	15:20	<0.10	N/A	<0.25	SK	5.5

[1]**Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (umhos/cm)	Analyst Initials
1	8.2	7.4	62	61	346	DF
2	7.2	9.9	74	85	451	DF
3	8.0	8.1	105	77	399	SK

DAILY RENEWAL CONDUCTIVITY**

Date		Sample #	Values are at Highest Dilution		Analyst
			Specific Conductivity as umhos/cm	Salinity (ppt)	
10/23	Lab H2O		395	0.2	LH
10/24	Lab H2O		403	0.2	JR/MM
10/25	Lab H2O		392	0.2	JLM
10/26	Lab H2O		396	0.2	SK
10/27	Lab H2O		406	0.2	LH
10/28	Lab H2O		419	0.2	LH
10/29	Lab H2O		423	0.2	CM
10/23	OUTFALL*	1	386	0.2	LH
10/24	OUTFALL*	1	410	0.2	JR/MM
10/25	OUTFALL*	1	398	0.2	JLM
10/26	OUTFALL*	2	416	0.2	SK
10/27	OUTFALL*	2	421	0.2	LH
10/28	OUTFALL*	3	444	0.3	LH
10/29	OUTFALL*	3	438	0.3	CM

**Conductivity is taken on the highest remaining effluent concentration used for test renewal, not necessarily 100%

Analysis Methods: Chlorine: Hanna Colorimeter #HI711, Ammonia: Hanna Colorimeter #HI733, Hardness: Hanna Photometer #HI96735, Alkalinity: Hanna Colorimeter #HI775, pH, DO, Conductivity: Thermo Versa Star Benchtop Meter

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Ceriodaphnia dubia**

Client: Nashville, City of

Lab ID: 70464

Facility: WPCP

Dilution Water(s): Synthetic Lab

Outfall: 001

Test Begin Date: October 23, 2018

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	12.5	25	50	69	100		
LH	10/23	Start	1	pH	7.9	8.0	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	9.1	8.9	8.9	8.9	8.6	8.6		
JR/MM M	10/24	24 Hr	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.3	8.0	8.3	8.3	8.3	8.3		
		Renew	1	pH	8.1	8.0	8.0	8.0	8.0	7.9		
				DO (mg/L)	8.1	8.1	8.1	8.1	8.2	8.2		
JLM	10/25	48 Hr	1	pH	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.1	8.0	8.0	7.9	7.9	7.9		
		Renew	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.0	8.3	8.3	8.4	8.4	8.5		
SK	10/26	72 Hr	1	pH	8.1	8.0	8.0	8.0	8.0	7.9		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.5	8.5	8.4		
		Renew	2	pH	7.9	8.0	8.0	7.9	7.9	7.9		
				DO (mg/L)	8.0	8.1	8.1	8.2	8.2	8.4		
LH	10/27	96 Hr	2	pH	8.0	8.0	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	8.2	8.1	8.1	8.1	8.1	8.1		
		Renew	2	pH	8.0	8.0	8.1	8.1	8.1	8.1		
				DO (mg/L)	8.5	8.6	8.7	8.7	8.9	8.9		
LH	10/28	120 Hr	2	pH	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.5	8.5	8.5	8.5	8.4	8.4		
		Renew	3	pH	7.9	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.5	8.5	8.5	8.5	8.5	8.5		
CM	10/29	144 Hr	3	pH	8.0	7.9	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	8.6	8.4	8.4	8.4	8.3	8.3		
		Renew	3	pH	8.0	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.1	8.3	8.2	8.2	8.3	8.3		
CM	10/30	168 Hr	3	pH	8.1	8.0	7.9	7.9	8.0	8.0		
		25 ± 1		DO (mg/L)	8.4	8.5	8.4	8.4	8.3	8.3		

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Pimephales promelas**

Client: Nashville, City of **Lab Number:** 70464

Facility: WPCP **Dilution Water(s):** Synthetic Lab

Outfall: 001 **Test Begin Date:** October 23, 2018

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	12.5	25	50	69	100		
LH	10/23	Start	1	pH	7.9	8.0	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	9.1	8.9	8.9	8.9	8.6	8.6		
JR/MM M	10/24	24 Hr	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.3	8.3	8.3	8.2	8.2	8.2		
		Renew	1	pH	8.1	8.0	8.0	8.0	8.0	7.9		
				DO (mg/L)	8.1	8.1	8.1	8.1	8.2	8.2		
JLM	10/25	48 Hr	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.4	8.2	8.2	8.1	8.1	8.0		
		Renew	1	pH	8.1	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.0	8.3	8.3	8.4	8.4	8.5		
SK	10/26	72 Hr	1	pH	8.0	7.9	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	7.9	7.9	7.9	7.9	7.9	7.9		
		Renew	2	pH	7.9	8.0	8.0	7.9	7.9	7.9		
				DO (mg/L)	8.0	8.1	8.1	8.2	8.2	8.4		
LH	10/27	96 Hr	2	pH	8.0	8.0	8.0	8.0	8.0	8.0		
		25 ± 1		DO (mg/L)	8.3	8.2	8.2	8.2	8.1	8.1		
		Renew	2	pH	8.0	8.0	8.1	8.1	8.1	8.1		
				DO (mg/L)	8.5	8.6	8.7	8.7	8.9	8.9		
LH	10/28	120 Hr	2	pH	8.1	8.0	8.0	8.0	7.9	7.9		
		25 ± 1		DO (mg/L)	8.9	8.8	8.9	8.9	8.7	8.7		
		Renew	3	pH	7.9	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.5	8.5	8.5	8.5	8.5	8.5		
CM	10/29	144 Hr	3	pH	7.9	7.9	7.9	7.9	8.0	8.0		
		25 ± 1		DO (mg/L)	8.1	7.9	7.9	7.9	7.9	7.9		
		Renew	3	pH	8.0	8.0	8.0	8.0	8.0	8.0		
				DO (mg/L)	8.1	8.3	8.2	8.2	8.3	8.3		
CM	10/30	168 Hr	3	pH	7.9	7.9	7.8	7.8	7.9	7.9		
		25 ± 1		DO (mg/L)	8.3	8.2	8.1	8.1	8.1	8.1		

Appendix B

Ceriodaphnia dubia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

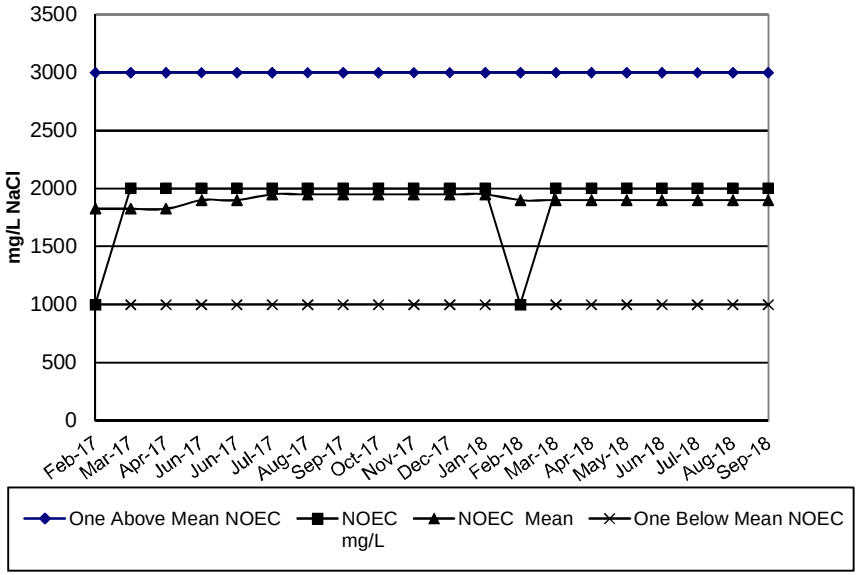
CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	3-Brood Chronic						
TEST NUMBER:	283						
PROJECT NUMBER:	70380 DOC						
START DATE:	9/4/2018						
START TIME:	12:01						
TOTAL NUMBER EXPOSED:	10 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	250	500	1000	2000	3000	4000
NUMBER DEAD PER CONCENTRATION:	1	0	0	1	4	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test REPRODUCTION: Steel's Many One Rank Test						

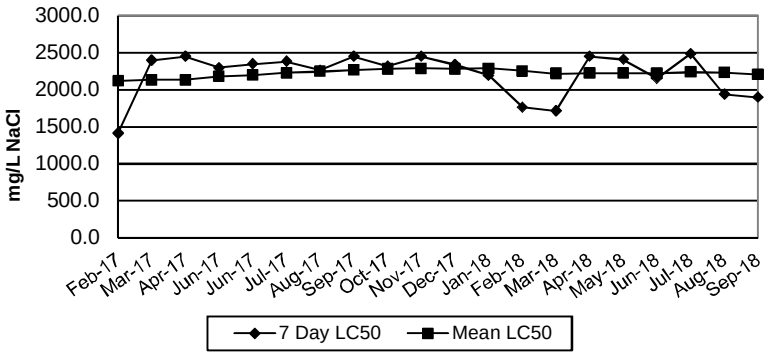
NOEC FOR SURVIVAL:	2000	mg/L
LOEC FOR SURVIVAL:	3000	mg/L
NOEC FOR REPRODUCTION:	500	mg/L
LOEC FOR REPRODUCTION:	1000	mg/L

PMSD: 20.9

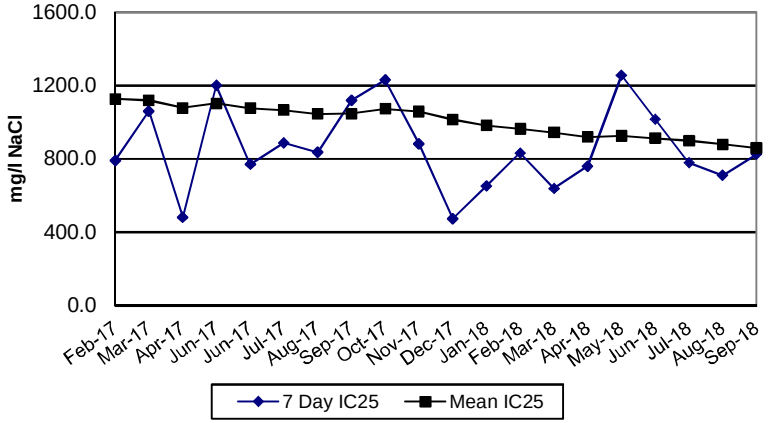
Ceriodaphnia Chronic Survival Control Chart



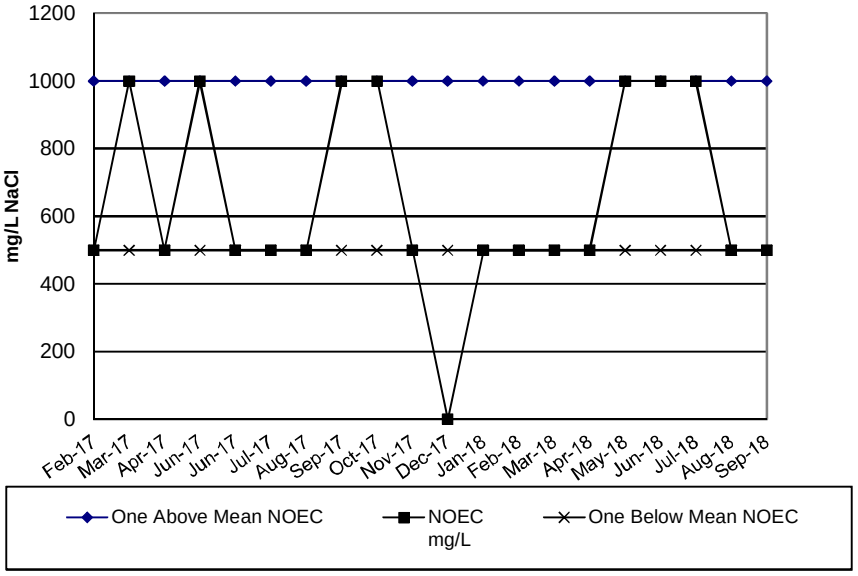
Ceriodaphnia 7-Day LC50



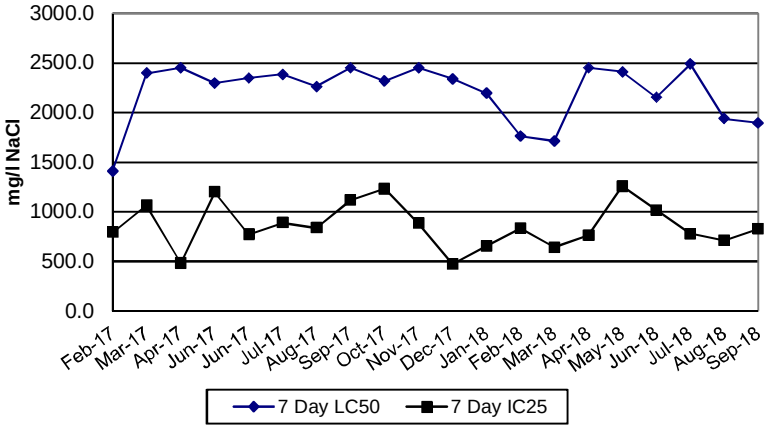
Ceriodaphnia 7-Day IC25



Ceriodaphnia Chronic Reproduction Control Chart



Ceriodaphnia 7-Day LC50 & IC25



Appendix B

Pimephales promelas

BIO-AQUATIC TESTING, INC.

Carrollton, TX

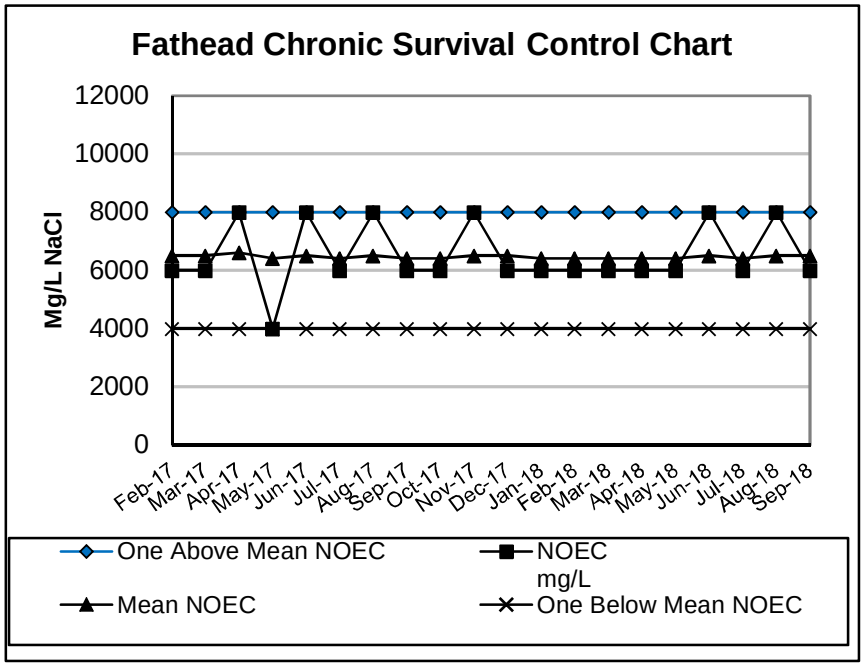
REFERENCE TOXICANTS

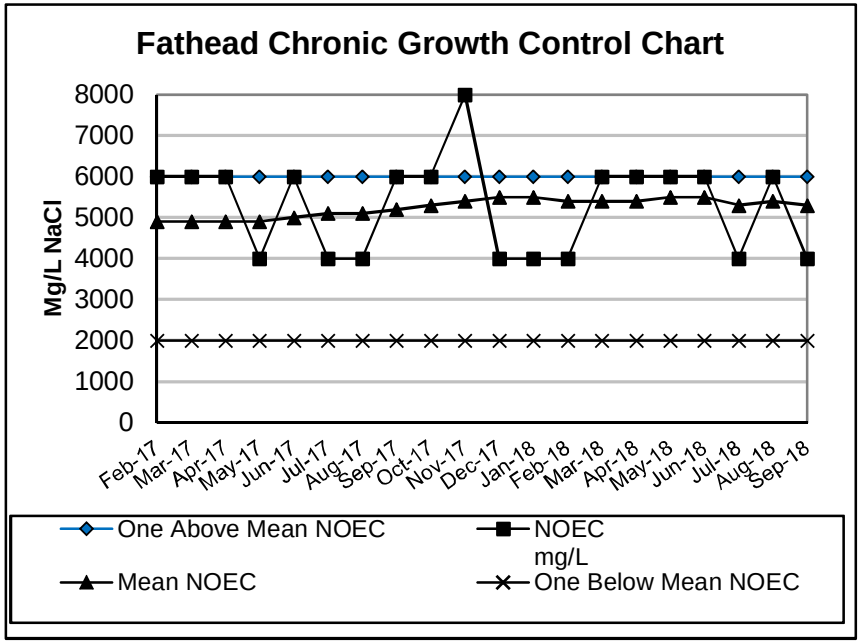
Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

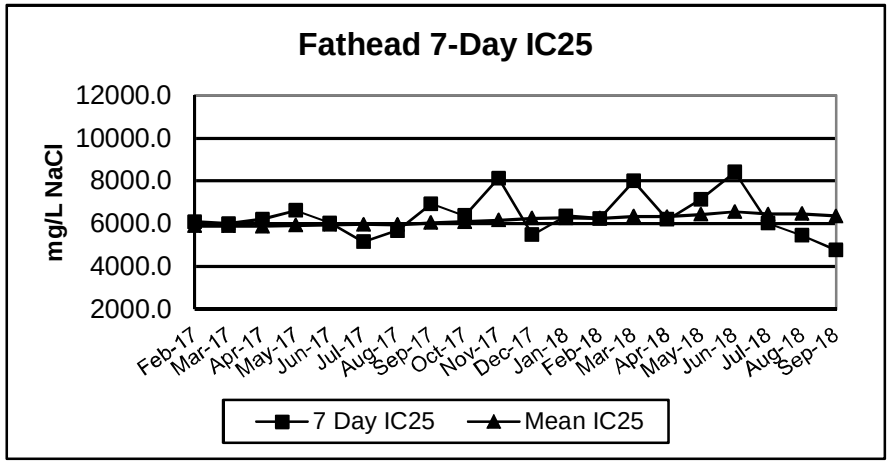
CHRONIC REFERENCE TOXICANT TEST RESULTS

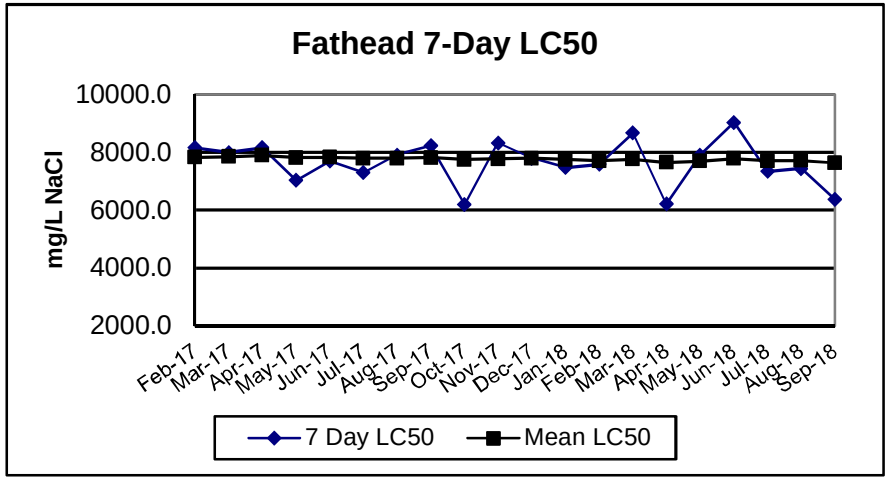
DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	7 Days						
TEST NUMBER:	323						
PROJECT NUMBER:	70384 DOC						
START DATE:	9/4/2018						
START TIME:	17:00						
TOTAL NUMBER EXPOSED:	40 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	1	2	2	15	33	40	40
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA and Dunnett's Test						
NOEC FOR SURVIVAL:	6000 mg/L						
LOEC FOR SURVIVAL:	8000 mg/L						
NOEC FOR GROWTH:	4000 mg/L						
LOEC FOR GROWTH:	6000 mg/L						

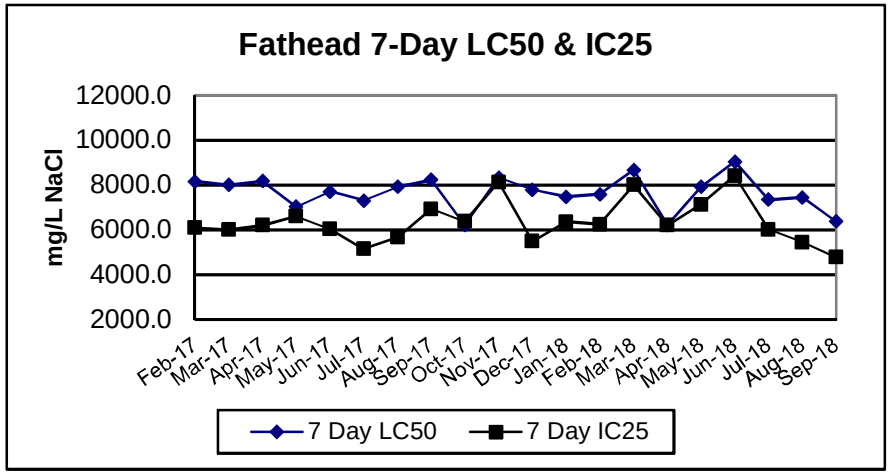
PMSD: 25.6


Fathead Chronic Survival Control Chart
Mg/L NaCl
12000
10000
8000
6000
4000
2000
0
Feb-17 Mar-17 Apr-17 May-17 Jun-17 Jul-17 Aug-17 Sep-17 Oct-17 Nov-17 Dec-17 Jan-18 Feb-18 Mar-18 Apr-18 May-18 Jun-18 Jul-18 Aug-18 Sep-18
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Fathead Chronic Growth Control Chart
Mg/L NaCl
8000
7000
6000
5000
4000
3000
2000
1000
0
Feb-17 Mar-17 Apr-17 May-17 Jun-17 Jul-17 Aug-17 Sep-17 Oct-17 Nov-17 Dec-17 Jan-18 Feb-18 Mar-18 Apr-18 May-18 Jun-18 Jul-18 Aug-18 Sep-18
One Above Mean NOEC
NOEC mg/L
Mean NOEC
One Below Mean NOEC


Fathead 7-Day IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-17 Mar-17 Apr-17 May-17 Jun-17 Jul-17 Aug-17 Sep-17 Oct-17 Nov-17 Dec-17 Jan-18 Feb-18 Mar-18 Apr-18 May-18 Jun-18 Jul-18 Aug-18 Sep-18
7 Day IC25
Mean IC25


Fathead 7-Day LC50
mg/L NaCl
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-17 Mar-17 Apr-17 May-17 Jun-17 Jul-17 Aug-17 Sep-17 Oct-17 Nov-17 Dec-17 Jan-18 Feb-18 Mar-18 Apr-18 May-18 Jun-18 Jul-18 Aug-18 Sep-18
7 Day LC50
Mean LC50


Fathead 7-Day LC50 & IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-17 Mar-17 Apr-17 May-17 Jun-17 Jul-17 Aug-17 Sep-17 Oct-17 Nov-17 Dec-17 Jan-18 Feb-18 Mar-18 Apr-18 May-18 Jun-18 Jul-18 Aug-18 Sep-18
7 Day LC50
7 Day IC25

APPENDIX C

LITERATURE REFERENCES

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.

U.S.E.P.A., 2012. Tropical Collector Urchin, *Tripneustes gratilla* (First Edition) U.S. Environmental Protection Agency, Office of Research and Development and Region 9, EPA-600-R-12-022.

U.S.E.P.A., 1995. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To West Coast Marine and Estuarine Organisms (First Edition) U.S. Environmental Protection Agency, EPA-600-R-95-136.

U.S.E.P.A., 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document, U.S. Environmental Protection Agency, Office of Wastewater, Washington D.C., EPA-833-R-10-004.

U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.

Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.

CHAIN-OF-CUSTODY SHEETS

Appendix D

BIO-AQUATIC TESTING, INC.

2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 70464

Please Review & Complete Sections A, B, C, & D.

Sample No: 70464 – ____

Revision 2 Effective Date 8/25/2017

Check Sample No. : ✓ First, ____ Second, or ____ Third.

P.O. No:

Client: Nashville, City of

Facility: WPCP

Permit No: GA0039365

Outfall: 001

Client Contact: Braelon Rice

Client Phone: 2295396876

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 12.5 25 50 69 100

To Ship the 1st Sample on: 10/8/2018

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex* (water flea)	*D. magna* (water flea)	*P. promelas* (minnow)	*Selenastrum* (green algae)	*M. beryllina* (minnow)	*Mysidopsis* (shrimp)
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: Annual Chronic Cerio/Fathead (DQ)

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	WWTP EFF	E	10/21/18	10/22/18	1000	0800	C	BR Braelon Rice	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	BR	10/22/18	820		10-23-18	1040
2						
3						

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date:	10-23	Time:	1040	By:		Temperature:	3.3 (C) IR#: 607
Chlorine:	<0.1 mg/l	Ammonia:	<0.25 mg/l	Int. Sal\Cond:	346 ppt/uS	Adj. Salinity	ppt
pH:	8.2	Hardness:	62 mg/l	Other			
DO:	7.4 mg/l	Alkalinity:	61 mg/l	Condition:			

Bio-Aquatic Lab ID: 70464

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Report Date: 11/16/2018 Revision 0

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 70464

Please Review & Complete Sections A, B, C, & D.

Sample No: 70464 -____ Revision 2 Effective Date 8/25/2017

Check Sample No. : ____ First, ✓ Second, or ____ Third.

P.O. No:

Client: Nashville, City of

Facility: WPCP

Permit No: GA0039365

Outfall: 001

Client Contact: Brandon Rice

Client Phone: 2295356576

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

To Ship the 1st Sample on: 10/8/2018

Concentration: 12.5 25 50 69 100

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex* (water flea)	*D. magna* (water flea)	*P. promelas* (minnow)	*Selenastrum* (green algae)	*M. beryllina* (minnow)	*Mysidopsis* (shrimp)
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: Annual Chronic Cerio/Fathead (DQ)

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	WWTP Eff	E	10/23/18	10/24/18	1045	0830	C	TBC Brandon Rice	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	TBC	10/24/18	900		10-24-18	0830
2						
3						

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 18-10-26 Time: 0830 By: C Temperature: 2.1 (C) IR#: 002

Chlorine: (0.1 mg/l Ammonia: (0.25 mg/l Int. Sal\Cond: 451 ppt/uS Adj. Salinity ppt

pH: 7.2 Hardness: 74 mg/l Other

DO: 9.9 mg/l Alkalinity: 85 mg/l Condition:

Bio-Aquatic Lab ID: 70464

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Report Date: 11/16/2018 Revision 0

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 70464

Please Review & Complete Sections A, B, C, & D.

Sample No: 70464 – ____ (Revision 2, Effective Date 9/25/2017)

Check Sample No. : ____ First, ✓ Second, or ____ Third.

P.O. No:

Client: Nashville, City of
Facility: WPCP
Permit No: GA0039365
Outfall: 001
Client Contact: Brandon Rice
Client Phone: 229-539-6976

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

To Ship the 1st Sample on: 10/8/2018

Concentration: 12.5 25 50 69 100

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: Annual Chronic Cerio/Fathead (DQ)

C. Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1 WWTP Eff	E	10/25/18	10/26/18	920 Am	815 Am	C	SA Steve Adams	1
2								
3								

D. Relinquished By:	Date	Time	Received By:	Date	Time
1 SA	10/26/18	845 Am		10-27-18	1520
2					
3					

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No
Dechlorinate Sample: ○ Yes ● No
Dilution Water: ○ Receiving Stream ● Synthetic Lab

Date: 10-27-18	Time: 1520	By:	Temperature: 5.5 (C)	IR#: 002	
Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 399 ppt/uS	Adj. Salinity ppt		
pH: 8.0	Hardness: 105 mg/l	Other			
DO: 8.1 mg/l	Alkalinity: 77 mg/l	Condition: Good			

Bio-Aquatic Lab ID: 70464
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Report Date: 11/16/2018 Revision 0

REGULATORY AGENCY TABLES

Appendix E

Table 1 (Sheet 1 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Nashville, City of - WPCP
Permit No.: GA0039365
Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	10/21/2018 @ 10:00	TO:	10/22/2018@ 08:00
	FROM:	10/23/2018 @ 10:45	TO:	10/24/2018@ 08:30
	FROM:	10/25/2018 @ 09:20	TO:	10/26/2018@ 08:15

Test Initiation: Time: 13:33 Date: 10/23/2018

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

NUMBER OF YOUNG PRODUCED PER ADULT AT TEST TERMINATION

	EFFLUENT CONCENTRATION (%)					
REPLICATE	0%	12.5 %	25 %	50 %	69 %	100
A	23	24	26	21	21	24
B	24	25	26	20	25	27
C	25	24	17	21	28	21
D	23	22	23	14	26	25
E	20	18	29	17	16	26
F	23	21	16	21	20	28
G	23	22	29	24	23	23
H	25	31	27	27	24	18
I	27	25	22	29	26	17
J	23	25	23	26	20	20
Surv. MEAN	23.6	23.7	23.8	22.0	22.9	22.9
Total MEAN	23.6	23.7	23.8	22.0	22.9	22.9
CV % [1]	7.7	14.3	19	20.8	15.8	16.5
PMSD	Acceptable Range 47 or Less					16.4 %

[1] Coefficient of Variation = (standard deviation/mean) x 100) Calculations are based on young of the surviving females. Males are designated (M), and dead females are designated (D) along with the number of neonates released prior to death. (E) anomalous value, spilled cup, or technician error.

Table 1 (Sheet 2 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Nashville, City of - WPCP

Permit No.: GA0039365

Outfall No.: 001

PERCENT SURVIVAL

	EFFLUENT CONCENTRATION (%)					
TIme of Reading	0%	12.5 %	25 %	50 %	69 %	100 %
24 HOURS	100.0	100.0	100.0	100.0	100.0	100.0
48 HOURS	100.0	100.0	100.0	100.0	100.0	100.0
7-DAY	100.0	100.0	100.0	100.0	100.0	100.0

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST
(with Bonferroni adjustment as appropriate for Sub-Lethality)
Is the mean number of young produced per adult significantly less (p=0.05) than the number of young per adult in the control for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (69) : _____YES _____X_____NO

If you report NO, enter a '0' on the DMR form for Parameter ***TWP3B****, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Sub-Lethal Pass/Fail.*

2. FISHER'S EXACT TEST (as appropriate for Lethality)

Is the mean survival at test end significantly less (p=0.05) than the control's survival for the % effluent corresponding to lethality?

CRITICAL DILUTION (69) : _____YES _____X_____NO

If you report NO, enter a '0' on the DMR form for Parameter ***TLP3B****, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

a. NOEC Survival = 100 % Effluent (Parameter TOP3B)

b. LOEC Survival = Q* % Effluent (Parameter TXP3B)

c. NOEC Reproduction = 100 % Effluent (Parameter TPP3B)

d. LOEC Reproduction = Q* % Effluent (Parameter TYP3B)

Q* refers to a value that is not calculable

Table 1 (Sheet 3 of 4)
BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Nashville, City of - WPCP
Permit No.: GA0039365
Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	10/21/2018 @ 10:00	TO:	10/22/2018@ 08:00
	FROM:	10/23/2018 @ 10:45	TO:	10/24/2018@ 08:30
	FROM:	10/25/2018 @ 09:20	TO:	10/26/2018@ 08:15

Test Initiation: Time: 13:37 Date: 10/23/2018

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

DATA TABLE FOR GROWTH OF *Pimephales promelas*

Effluent Concentration	Average Dry Weight in milligrams (mg) per replicate					Mean Dry Weight (mg)	CV % [1]
	A	B	C	D	E		
0%	0.355	0.467	0.406	0.491	0.549	0.453	16.60
12.5 %	0.461	0.456	0.415	0.440	0.416	0.438	4.95
25 %	0.369	0.550	0.489	0.437	0.499	0.469	14.68
50 %	0.398	0.460	0.340	0.514	0.295	0.401	21.95
69 %	0.446	0.554	0.511	0.416	0.451	0.476	11.72
100 %	0.520	0.491	0.445	0.529	0.461	0.489	7.41
PMSD	Acceptable Range 30 or Less					20.4 %	

[1] Coefficient of Variation = (standard deviation/mean) x 100) ?= cannot be calculated due to 100% mortality or lab exception

DATA TABLE FOR SURVIVAL OF *Pimephales promelas*

Effluent Concentration	Percent Survival per replicate					Average % Survival			CV % [1]
	A	B	C	D	E	24 Hours	48 Hours	7-Day	
0%	75	87.5	75	100	87.5	100	100	85	12.30
12.5 %	100	87.5	100	100	75	100	100	92.5	12.09
25 %	87.5	100	100	100	87.5	100	100	95	7.21
50 %	100	87.5	100	100	75	100	100	92.5	12.09
69 %	87.5	100	87.5	87.5	100	100	100	92.5	7.40
100 %	100	100	100	87.5	100	100	100	97.5	5.73

Table 1 (Sheet 4 of 4)

BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Nashville, City of - WPCP

Permit No.: GA0039365

Outfall No.: 001

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST (with Bonferroni adjustment as appropriate for Sub-Lethality)

Is the mean dry weight at 7 days significantly less (p=0.05) than the control's mean dry weight for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (69) : _____YES ____X____NO

If you report NO, enter a '0' on the DMR form for Parameter ***TWP6C****, other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Sub-Lethal Pass/Fail.*

2. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST (as appropriate for Lethality) Is the survival at 7 days significantly less (p=0.05) than the control's survival for % effluent corresponding to lethality?

CRITICAL DILUTION (69) : _____YES ____X____NO

If you report NO, enter a '0' on the DMR form for Parameter ***TLP6C****, other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

			For DMR Form:
a.	NOEC Survival =	100 % Effluent	(Parameter TOP6C)
b.	LOEC Survival =	Q* % Effluent	(Parameter TXP6C)
c.	NOEC Growth =	100 % Effluent	(Parameter TPP6C)
d.	LOEC Growth =	Q* % Effluent	(Parameter TYP6C)

Q* refers to a value that is not calculable